Figure 1

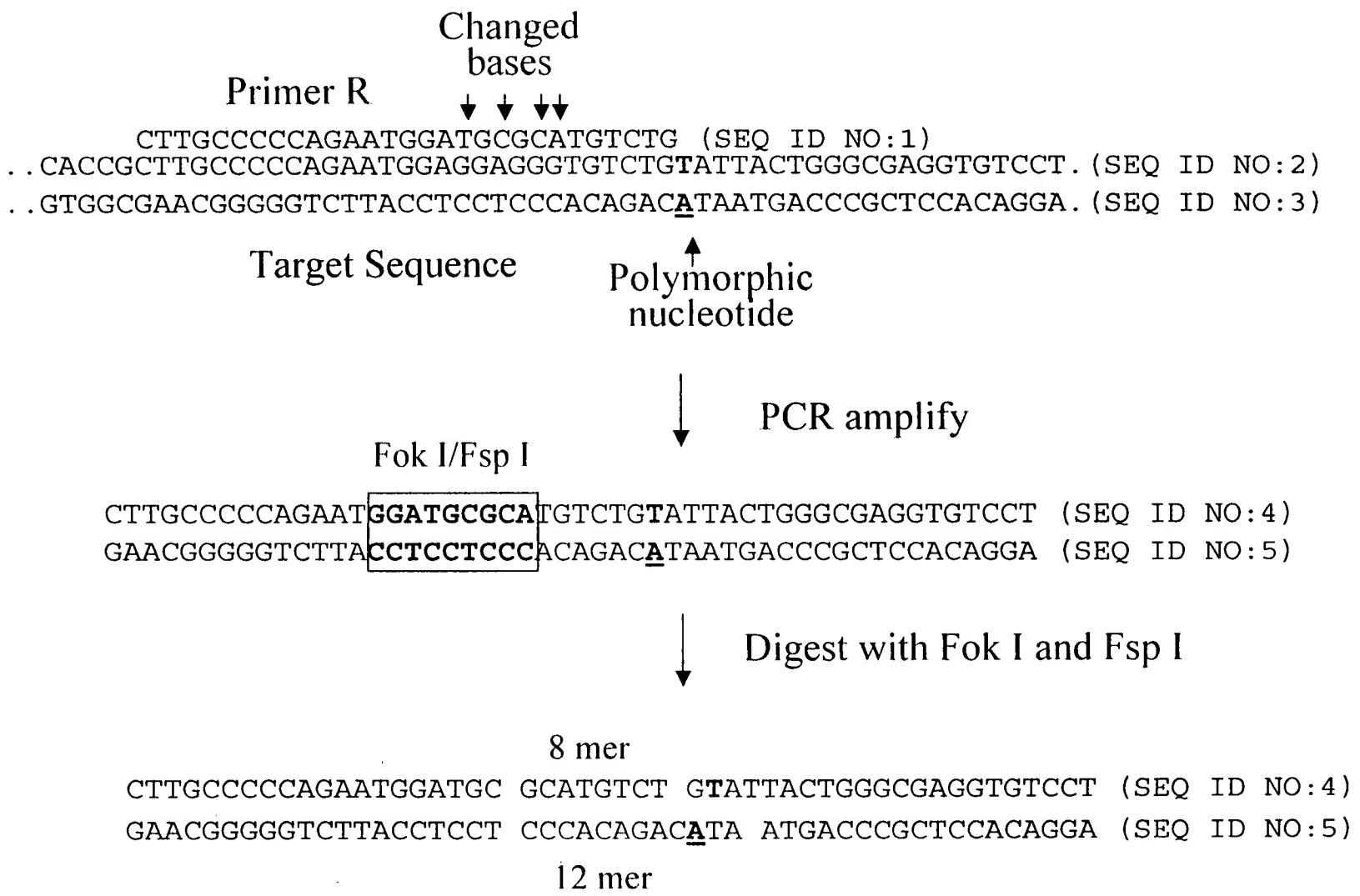

Changed bases
Primer R
CTTGCCCCCAGAATGGATGCGCATGTCTG (SEQ ID NO:1)
..CACCGCTTGCCCCCAGAATGGAGGAGGGTGTCTGTATTACTGGGCGAGGTGTCCT.(SEQ ID NO:2)
..GTGGCGAACGGGGGTCTTACCTCCTCCCACAGACATAATGACCCGCTCCACAGGA.(SEQ ID NO:3)
Target Sequence
Polymorphic nucleotide
PCR amplify
Fok I/Fsp I
CTTGCCCCCAGAATGGATGCGCATGTCTGTATTACTGGGCGAGGTGTCCT (SEQ ID NO:4)
GAACGGGGGTCTTACCTCCTCCCACAGACATAATGACCCGCTCCACAGGA (SEQ ID NO:5)
Digest with Fok I and Fsp I
8 mer
CTTGCCCCCAGAATGGATGC GCATGTCT GTATTACTGGGCGAGGTGTCCT (SEQ ID NO:4)
GAACGGGGGTCTTACCTCCT CCCACAGACATA ATGACCCGCTCCACAGGA (SEQ ID NO:5)
12 mer

Figure 2

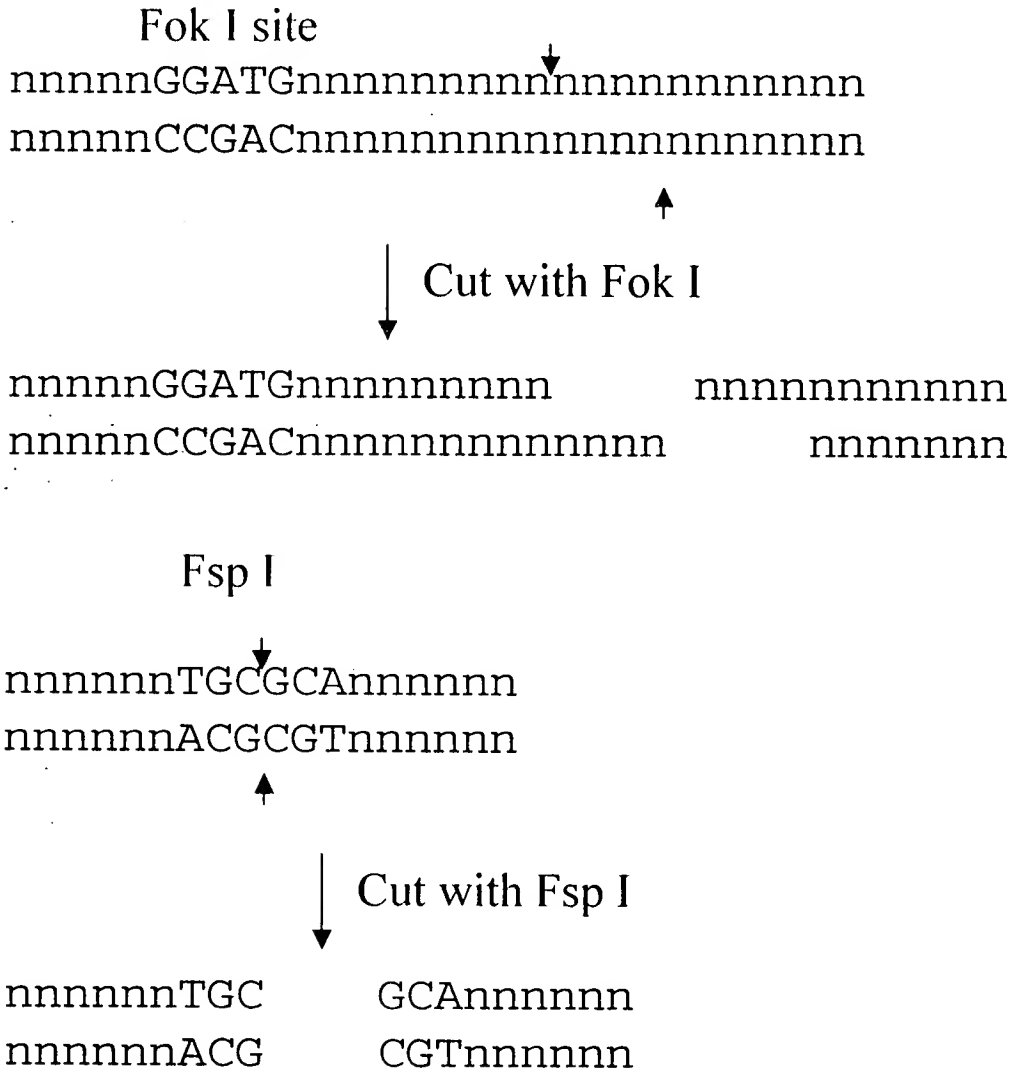

Fok I site
nnnnnGGATGnnnnnnnnnnnnnnnnnnn
nnnnnCCGACnnnnnnnnnnnnnnnnnnn
Cut with Fok I
nnnnnGGATGnnnnnnnnn
nnnnnnnnnn
nnnnnCCGACnnnnnnnnnnnnn
nnnnnnn
Fsp I
nnnnnnTGCGCAnnnnnn
nnnnnnACGCGTnnnnnn
Cut with Fsp I
nnnnnnTGC
GCAnnnnnn
nnnnnnACG
CGTnnnnnn

Figure 3

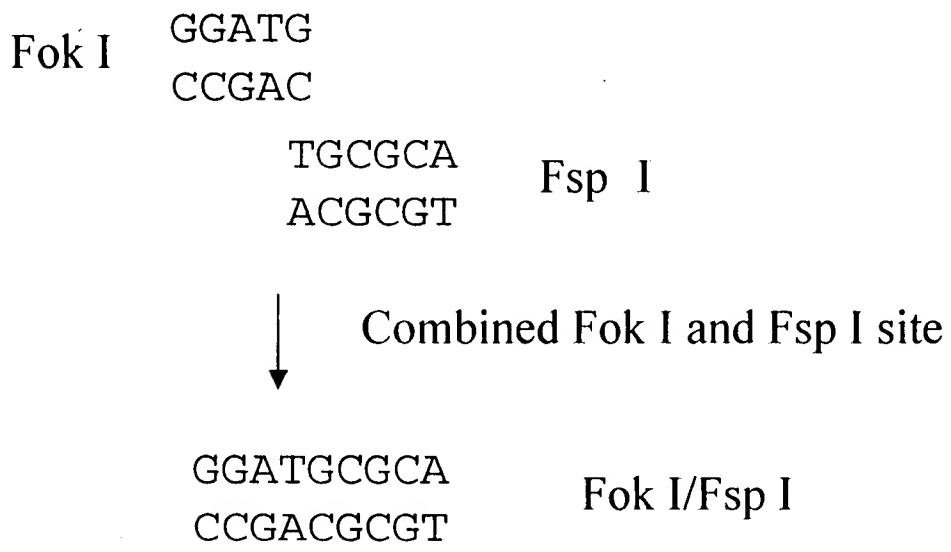

Fok I
GGATG
CCGAC
TGCGCA
ACGCGT
Fsp I
Combined Fok I and Fsp I site
GGATGCGCA
CCGACGCGT
Fok I/Fsp I

Figure 4. Restriction Enzyme Genotyping

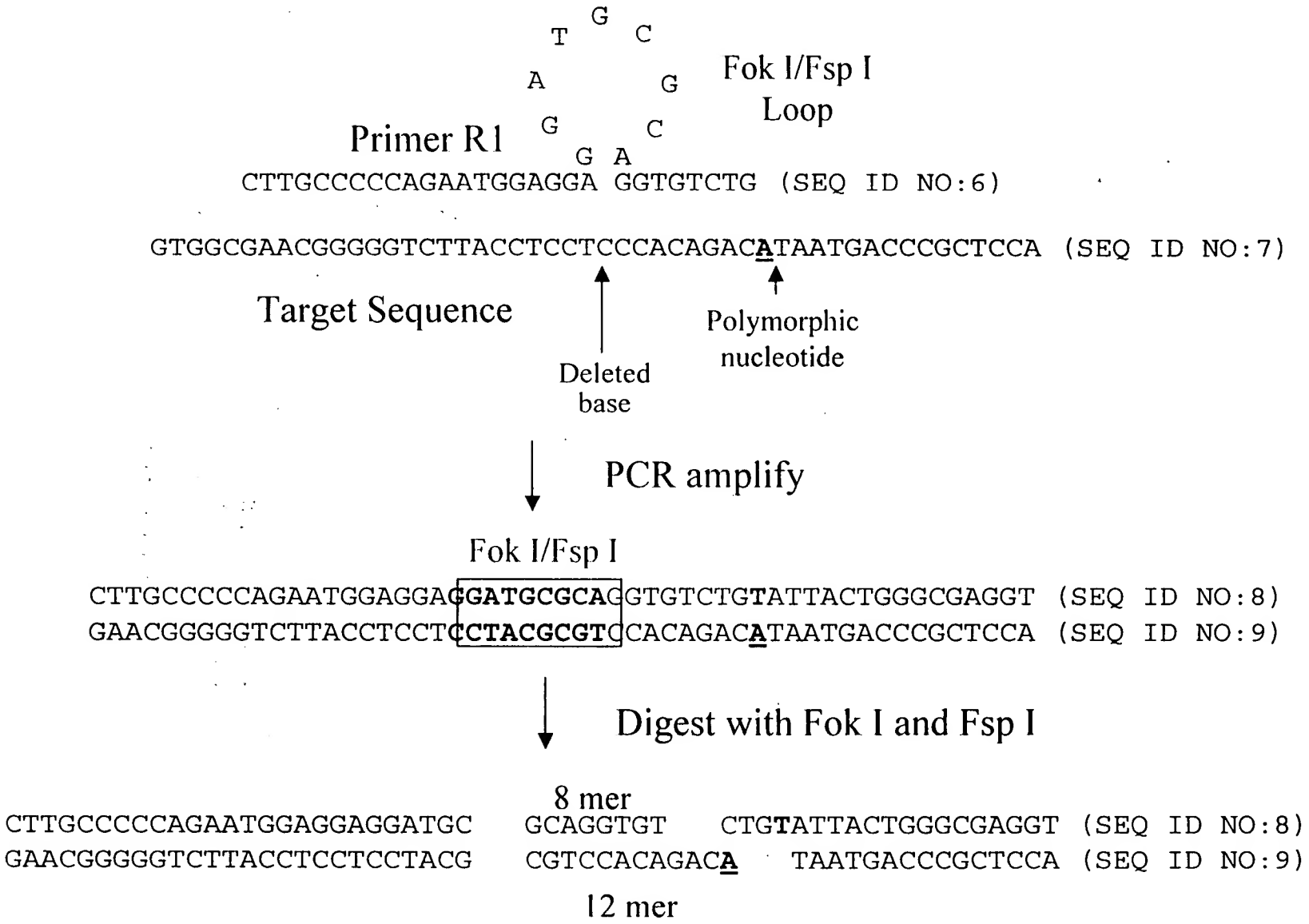


Figure 5. Introduction of Bsg I and Pvu II sites during PCR by loop followed by endonuclease digestion.

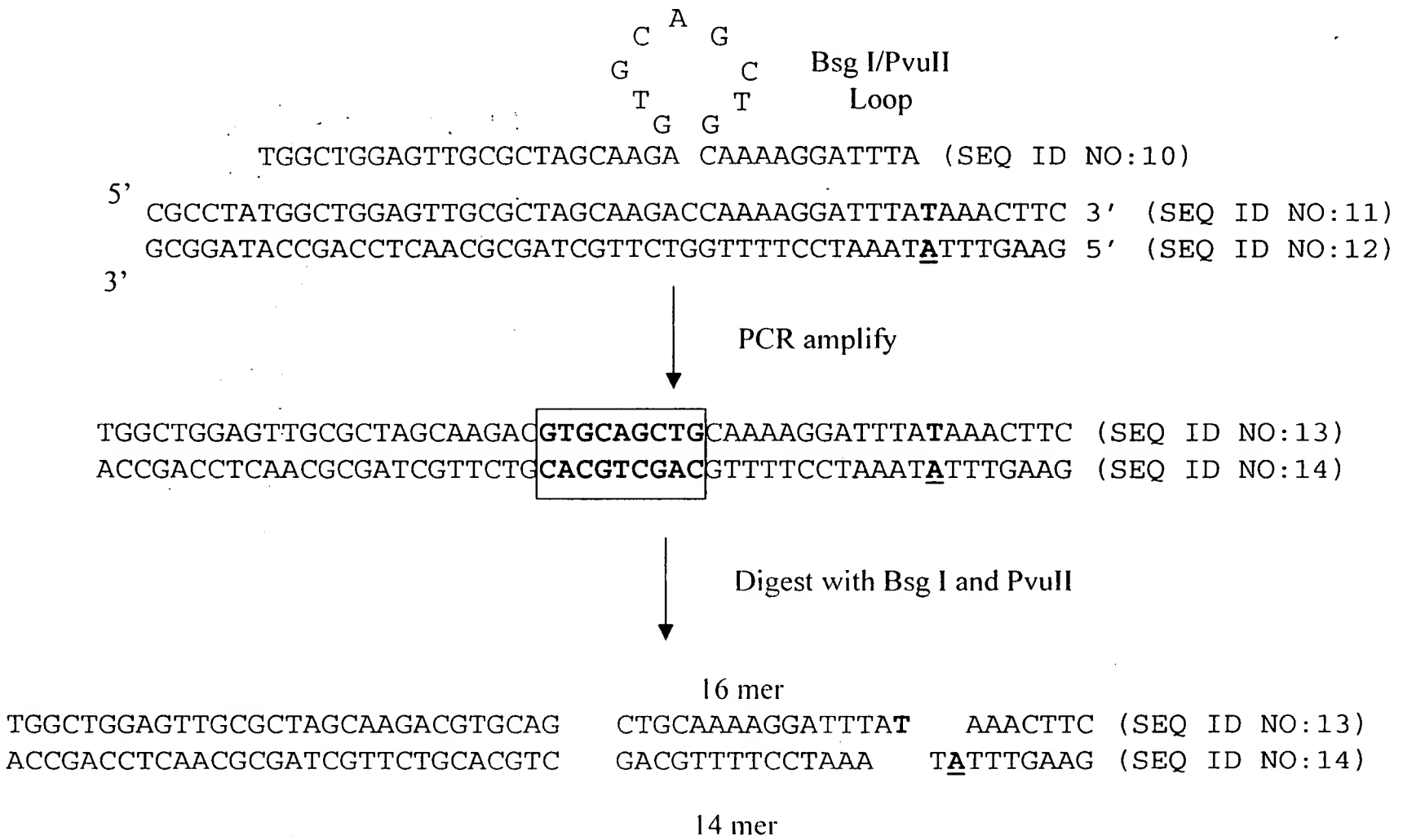
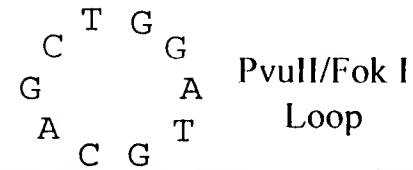


Figure 6. Introduction of Fok I and Pvu II sites during PCR by loop followed by endonuclease digestion



TGGCTGGAGTTGCGCTAGCAAGACCA AAGGATTTA (SEQ ID NO:15)

5′ CGCCTATGGCTGGAGTTGCGCTAGCAAGACCAAAAGGATTTA**T**AAACTTC 3′(SEQ ID NO:16)
3′ GCGGATACCGACCTCAACGCGATCGTTCTGGTTTTCCTAAAT**A**TTTGAAG 5′(SEQ ID NO:17)

PCR amplify

5′CGCCTATGGCTGGAGTTGCGCTAGCAAGACCA**CAGCTGGATG**AAGGATTTA**T**AAACTTC 3′ (SEQ ID NO:18)
3′GCGGATACCGACCTCAACGCGATCGTTCTGGT**GTCGACCTAC**TTCCTAAAT**A**TTTGAAG 5′ (SEQ ID NO:19)

Digest with Pvu II and Fok I

16 mer

CGCCTATGGCTGGAGTTGCGCTAGCAAGACCACAG CTGGATGAAGGATTTA **T**AAACTTC (SEQ ID NO:18)
GCGGATACCGACCTCAACGCGATCGTTCTGGTGTC GACCTACTTCCTAAAT**A**TTT GAAG (SEQ ID NO:19)

20 mer

Figure 7

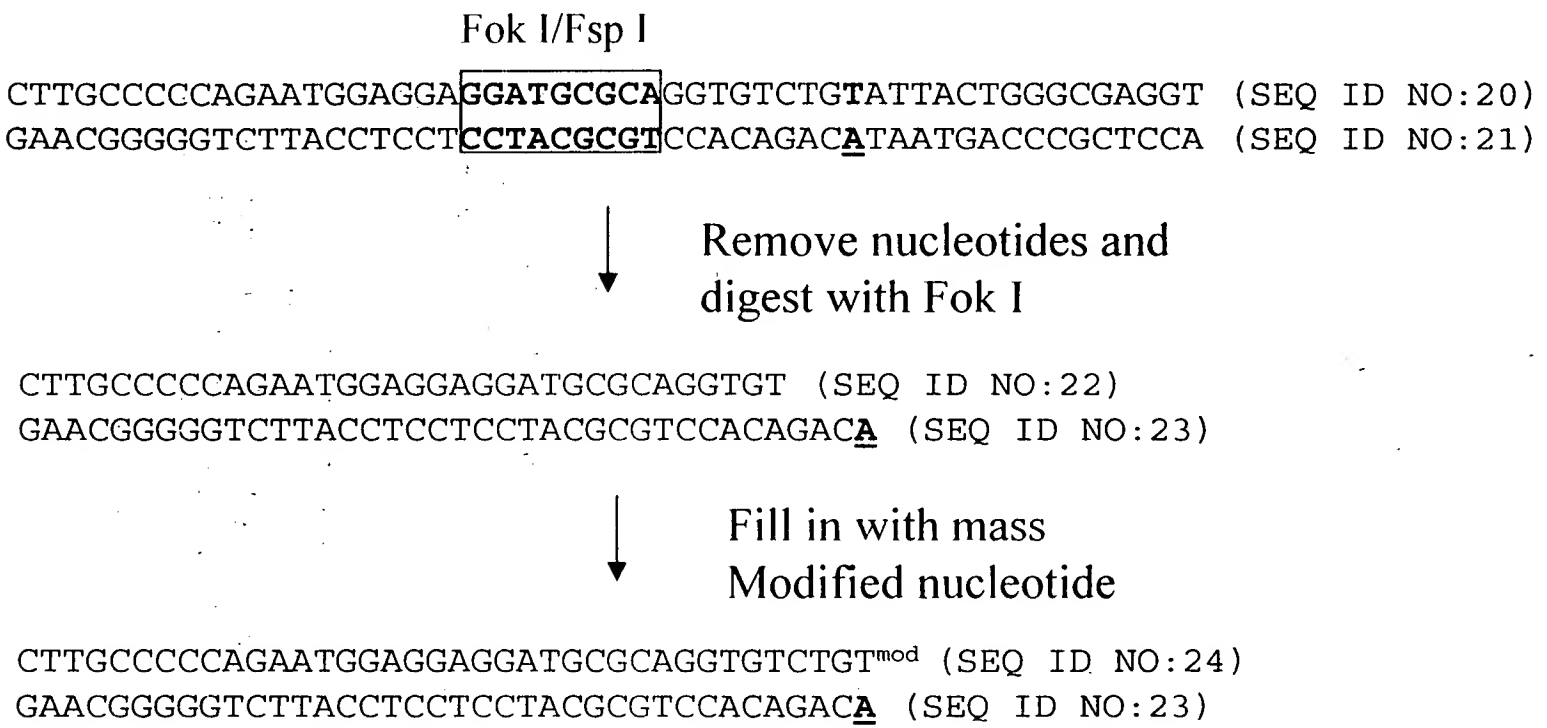

Fok I/Fsp I
CTTGCCCCCAGAATGGAGGAGGATGCGCAGGTGTCTGTATTACTGGGCGAGGT (SEQ ID NO:20)
GAACGGGGGTCTTACCTCCTCCTACGCGTCCACAGACATAATGACCCGCTCCA (SEQ ID NO:21)
Remove nucleotides and
digest with Fok I
CTTGCCCCCAGAATGGAGGAGGATGCGCAGGTGT (SEQ ID NO:22)
GAACGGGGGTCTTACCTCCTCCTACGCGTCCACAGACA (SEQ ID NO:23)
Fill in with mass
Modified nucleotide
CTTGCCCCCAGAATGGAGGAGGATGCGCAGGTGTCTGTmod (SEQ ID NO:24)
GAACGGGGGTCTTACCTCCTCCTACGCGTCCACAGACA (SEQ ID NO:23)

Figure 8

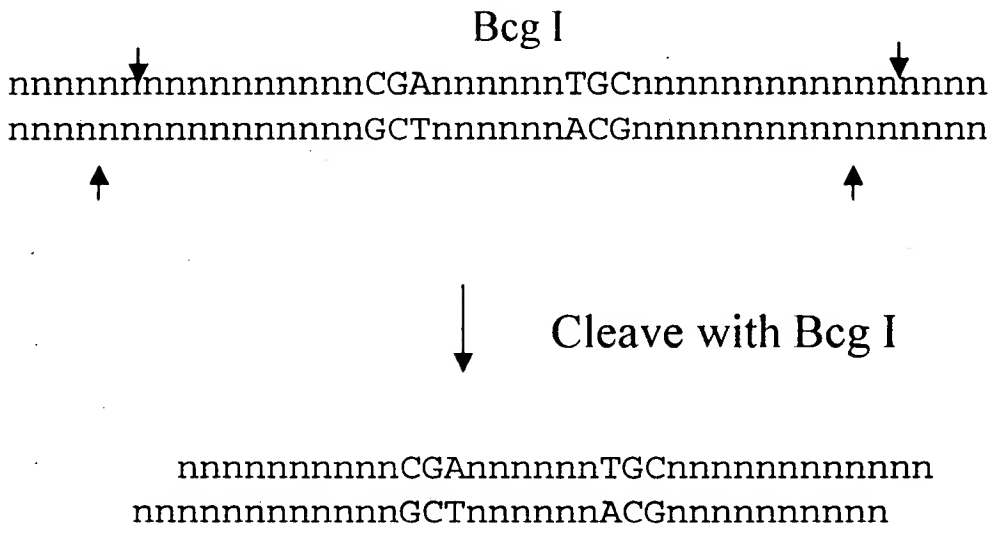

Bcg I
nnnnnnnnnnnnnnnnCGAnnnnnnTGCnnnnnnnnnnnnnnnn
nnnnnnnnnnnnnnnnGCTnnnnnnACGnnnnnnnnnnnnnnnn
Cleave with Bcg I
nnnnnnnnnnCGAnnnnnnTGCnnnnnnnnnnnn
nnnnnnnnnnnnGCTnnnnnnACGnnnnnnnnnn

Figure 9

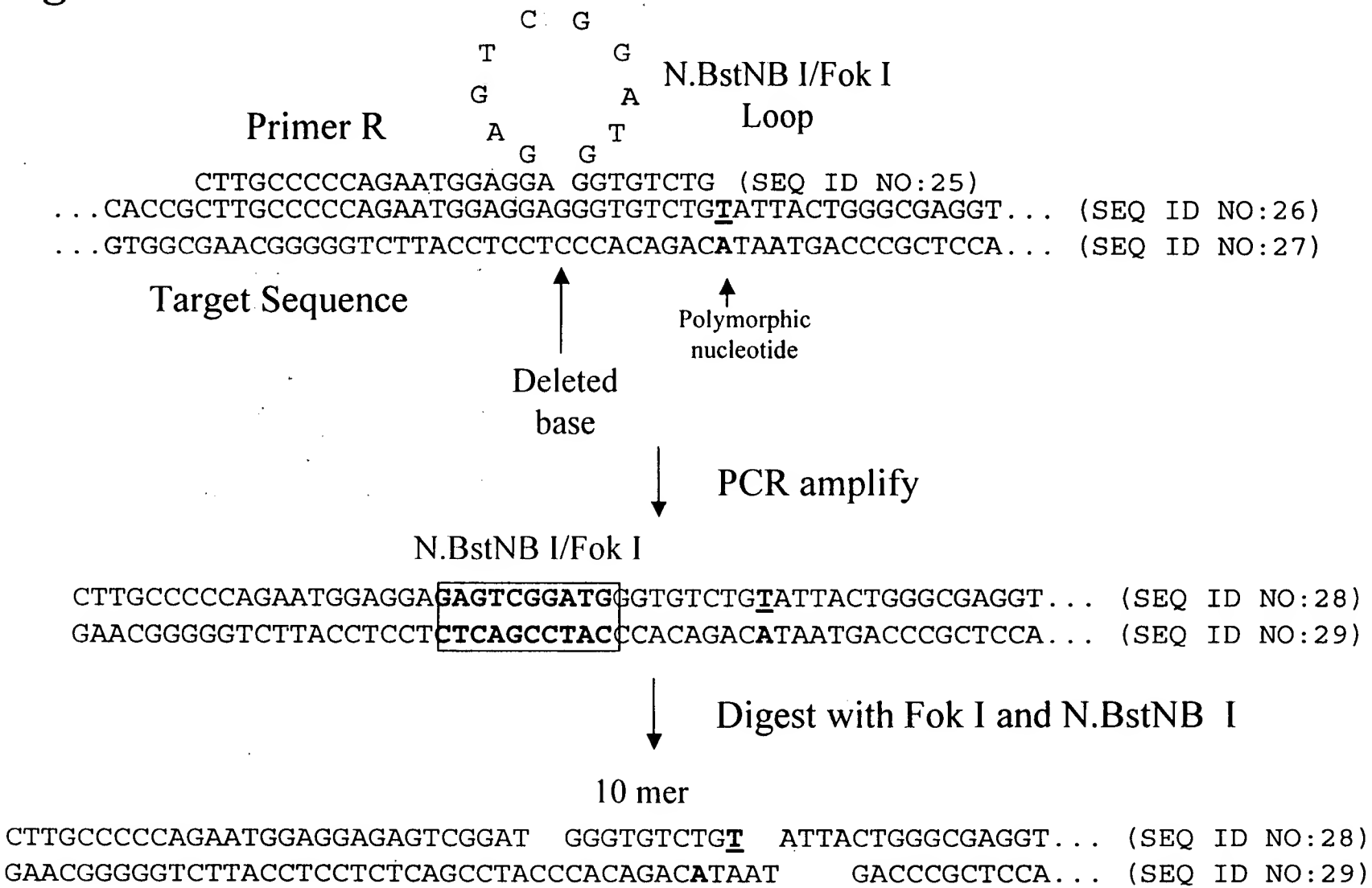

C G
T G
G A
A T
G G
Primer R
N.BstNB I/Fok I
Loop
CTTGCCCCCAGAATGGAGGA GGTGTCTG (SEQ ID NO:25)
...CACCGCTTGCCCCCAGAATGGAGGAGGGTGTCTGTATTACTGGGCGAGGT... (SEQ ID NO:26)
...GTGGCGAACGGGGGTCTTACCTCCTCCCACAGACATAATGACCCGCTCCA... (SEQ ID NO:27)
Target Sequence
Polymorphic nucleotide
Deleted base
PCR amplify
N.BstNB I/Fok I
CTTGCCCCCAGAATGGAGGAGAGTCGGATGGGTGTCTGTATTACTGGGCGAGGT... (SEQ ID NO:28)
GAACGGGGGTCTTACCTCCTCTCAGCCTACCCACAGACATAATGACCCGCTCCA... (SEQ ID NO:29)
Digest with Fok I and N.BstNB I
10 mer
CTTGCCCCCAGAATGGAGGAGAGTCGGAT GGGTGTCTGT ATTACTGGGCGAGGT... (SEQ ID NO:28)
GAACGGGGGTCTTACCTCCTCTCAGCCTACCCACAGACATAAT GACCCGCTCCA... (SEQ ID NO:29)

Figure 10

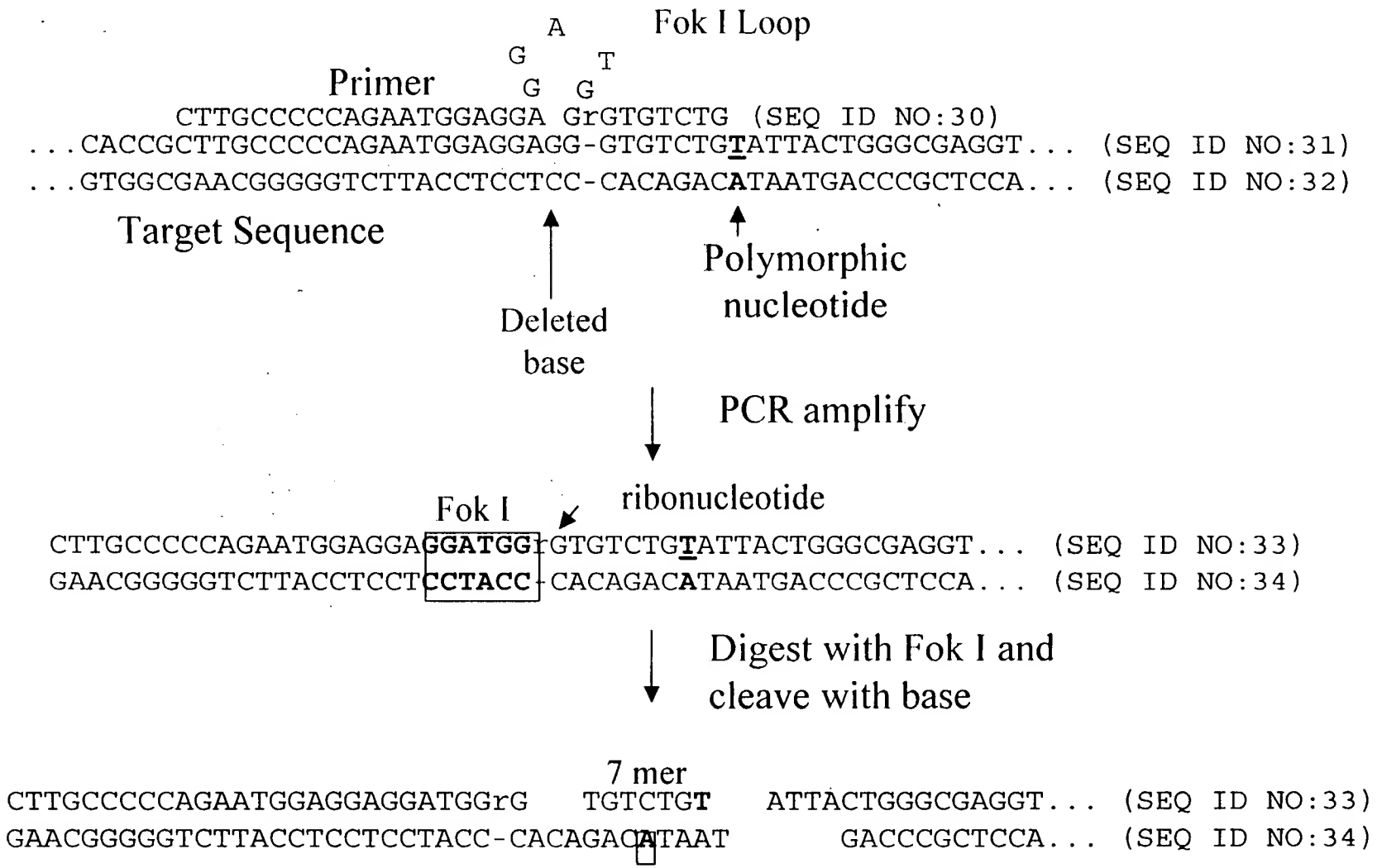

A
Fok I Loop
G
T
Primer
G G
CTTGCCCCCAGAATGGAGGA GrGTGTCTG (SEQ ID NO:30)
...CACCGCTTGCCCCCAGAATGGAGGAGG-GTGTCTGTATTACTGGGCGAGGT... (SEQ ID NO:31)
...GTGGCGAACGGGGGTCTTACCTCCTCC-CACAGACATAATGACCCGCTCCA... (SEQ ID NO:32)
Target Sequence
Deleted base
Polymorphic nucleotide
PCR amplify
Fok I
ribonucleotide
CTTGCCCCCAGAATGGAGGAGGATGGrGTGTCTGTATTACTGGGCGAGGT... (SEQ ID NO:33)
GAACGGGGGTCTTACCTCCTCCTACC-CACAGACATAATGACCCGCTCCA... (SEQ ID NO:34)
Digest with Fok I and cleave with base
7 mer
CTTGCCCCCAGAATGGAGGAGGATGGrG TGTCTGT ATTACTGGGCGAGGT... (SEQ ID NO:33)
GAACGGGGGTCTTACCTCCTCCTACC-CACAGACATAAT GACCCGCTCCA... (SEQ ID NO:34)

Figure 11. Methods for haplotyping based on physical allele separation

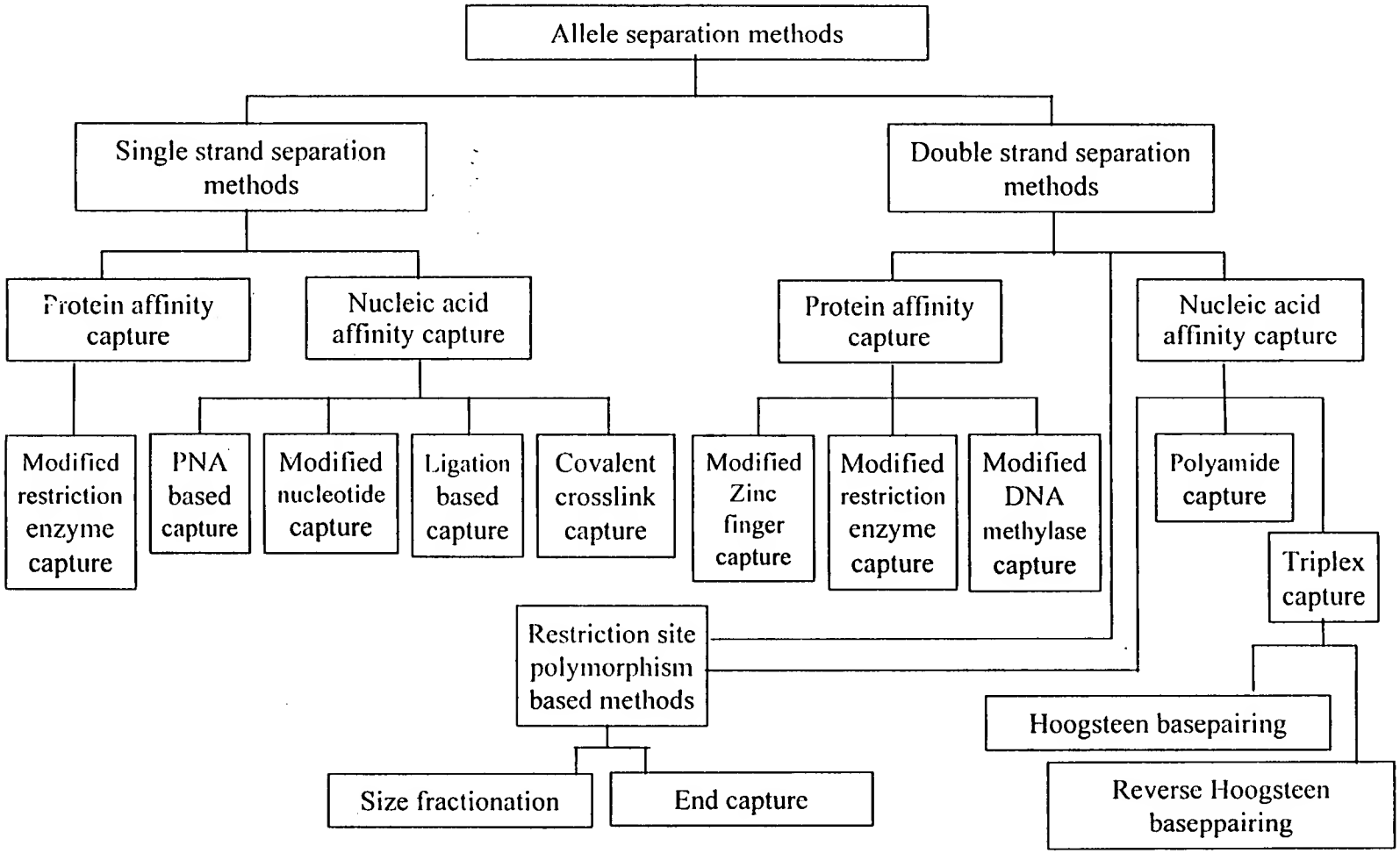
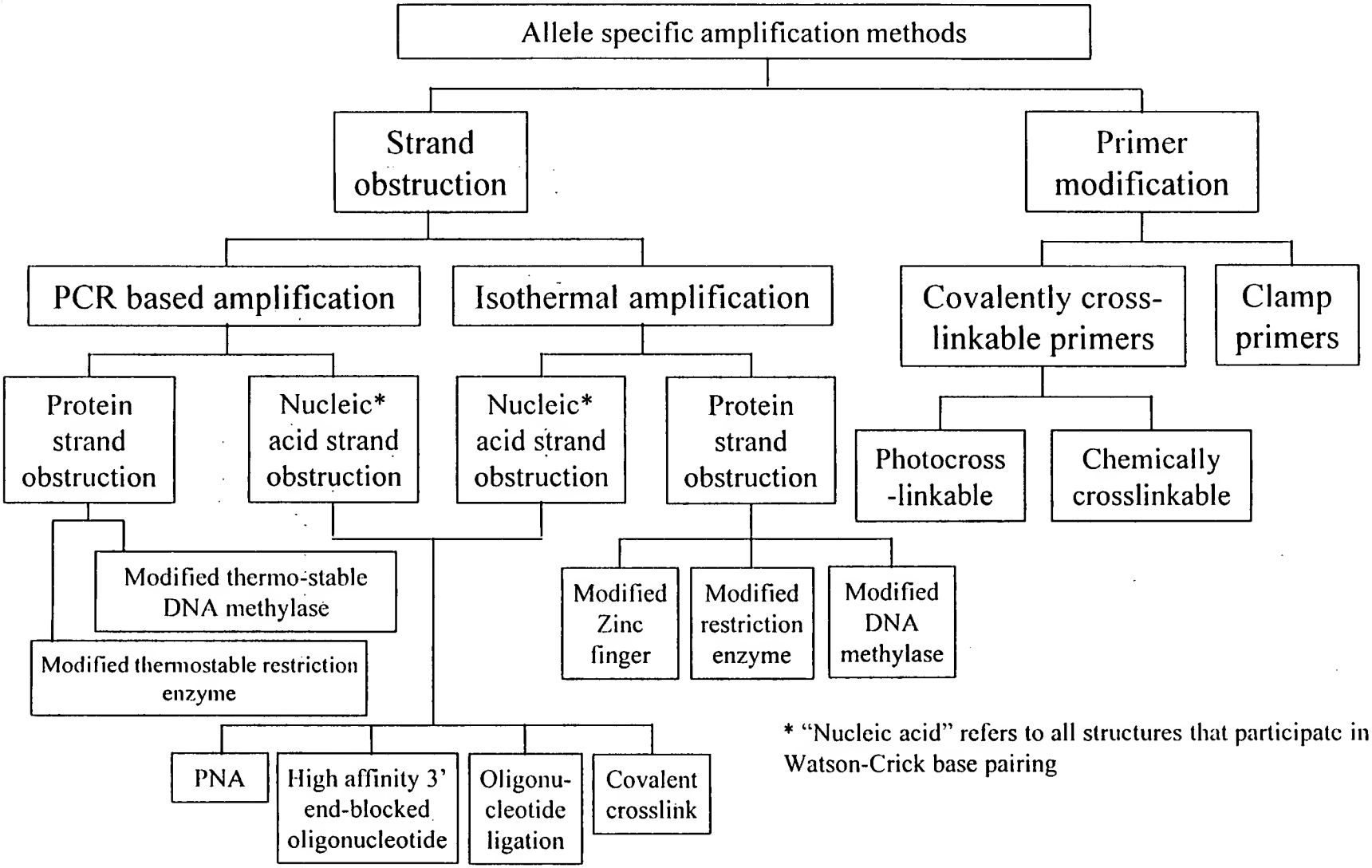


Figure 12. Methods for haplotyping based on allele specific amplification



* "Nucleic acid" refers to all structures that participate in Watson-Crick base pairing

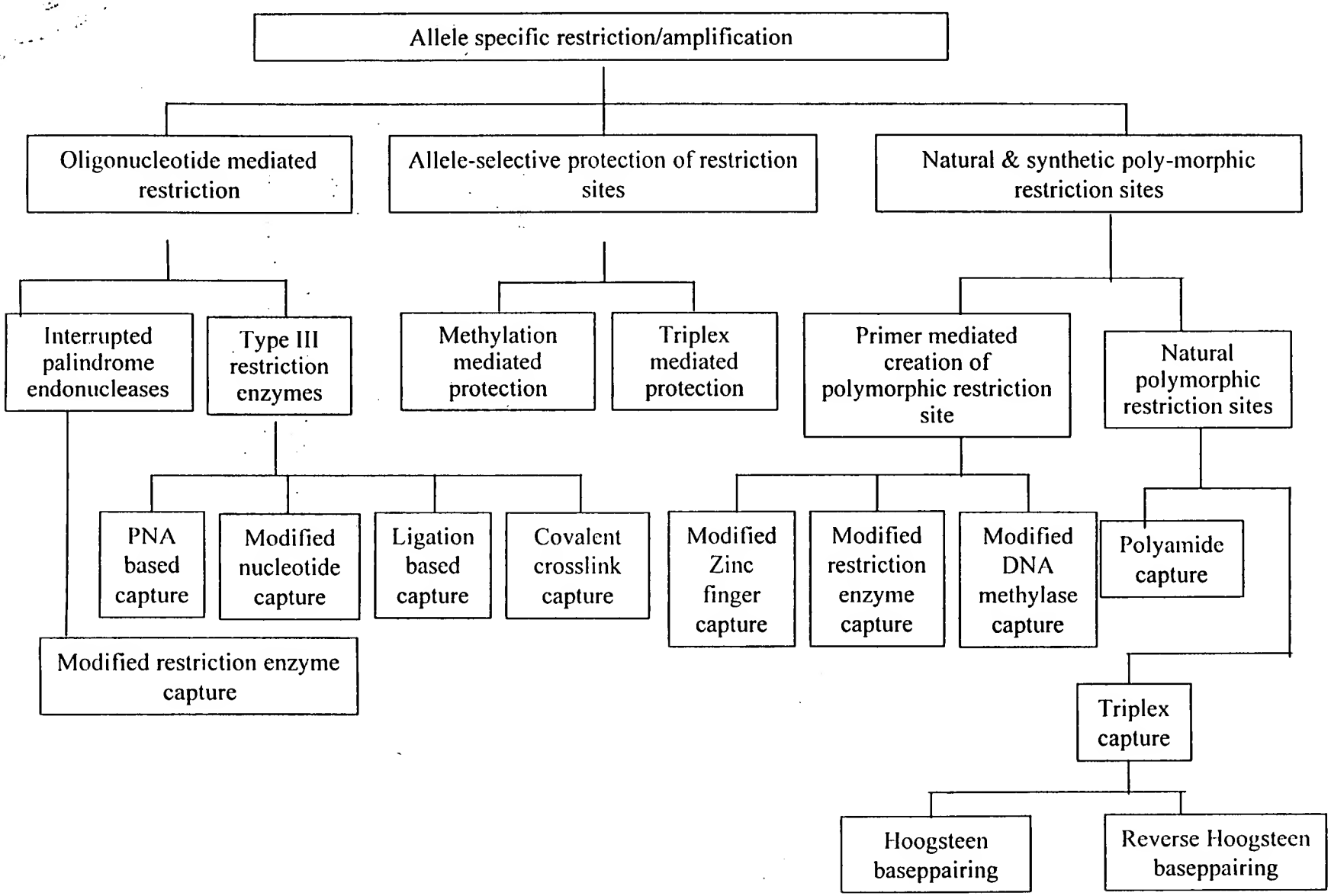



Figure 13. Methods for haplotyping based on allele specific restriction